

AMENDMENTS TO THE SPECIFICATION

Please amend paragraphs [0041]-[0044], [0046], [0056], [0077], [0097], [00125] and [00187]-[00191] as follows:

[0041] Figure 2 illustrates a crystal of the complex of the catalytic domain of CatS (residues 115-331 of SEQ. ID No. 1)- ~~with E64 complex~~.

[0042] Figure 3 lists a set of atomic structure coordinates for the catalytic domain of CatS (residues 115-331 of SEQ. ID No. 1) as derived by X-ray crystallography from a crystal that comprises the protein. The reference numbers in column E of Figure 3 correspond to residue numbers of SEQ. ID No. 3. The following abbreviations are used in Figure 3: “X, Y, Z” crystallographically define the atomic position of the element measured; “B” is a thermal factor that measures movement of the atom around its atomic center; “Occ” is an occupancy factor that refers to the fraction of the molecules in which each atom occupies the position specified by the coordinates (a value of “1” indicates that each atom has the same conformation, i.e., the same position, in all molecules of the crystal).

[0043] Figure 4 is a schematic diagram highlighting the secondary structural elements of the catalytic domain of CatS (residues 115-331 of SEQ. ID No. 1) and the binding region of E64.

[0044] Figure 5A illustrates a surface accessible representation of the molecular surface of the catalytic domain of CatS (residues 115-331 of SEQ. ID No. 1) showing the long binding pocket with E64 bound in the active site, based on the structure coordinates shown in Figure 3, chain A.

[0046] Figure 6 illustrates a system that may be used to carry out instructions for displaying a crystal structure of the catalytic domain of CatS (residues 115-331 of SEQ. ID No. 1) encoded on a storage medium.

[0056] For example, based on the crystal structure, applicants have determined that ~~Cat S~~ CatS has an elongated binding ~~pockets~~ pocket capable of binding to an E64 molecule (L-trans-epoxysuccinyl-leucylamido(4-guanidino)butane). Figures 5A and 5B illustrate an E64 molecule bound in the CatS binding pocket.

[0077] The gene encoding CatS can be isolated from RNA, cDNA or cDNA libraries. In this case, the portion of the gene encoding amino acid residues 1-331 of SEQ ID No. 1 was isolated and is shown as SEQ. I.D. No. 2.

[0097] By performing submicroliter volume sized crystallization experiments, as detailed in U.S. Patent No. 6,296,673, effective crystallization conditions for forming crystals of a CatS-E64 complex were obtained. In order to accomplish this, systematic broad screen crystallization trials were performed on a CatS-E64 complex using the sitting drop technique. Over 1000 individual trials were performed in which pH, temperature and precipitants were varied. In each experiment, a 100nL mixture of CatS-E64 complex and precipitant was placed on a platform positioned over a well containing ~~100nL~~ 100μL of the precipitating solution. Precipitate and crystal formation was detected in the sitting drops. Fine screening was then carried out for those crystallization conditions that appeared to produce precipitate and/or crystal in the drops.

[00125] The refined crystal structure of CatS-E64, determined according to the present invention, contains amino acids residues ~~115~~ 114-331 as numbered according to SEQ. ID No. 1 (based on the coordinates of Figure 3) and a bound E64 molecule. A total of 627 water molecules were included.

[00187] The portion of the gene encoding residues ~~1~~ 114-331 (from SEQ. ID No. 1) ~~which corresponds to the entire sequence of human CatS~~ was amplified by PCR and cloned into a modified pFastbac vector (Invitrogen) with a Glycine-6x-histidine tag at the C-terminus. ~~This DNA sequence is presented in Figure 1 as SEQ. ID No. 2.~~

[00188] Expression in this vector generated a fusion of CatS residues ~~1-340~~ 114-331 of SEQ. ID No. 1 with a C-terminal Glycine-6x-histidine tag, the amino acid sequence of which is shown in Figure 1 as SEQ. ID. No. 4 3. Recombinant baculoviruses incorporating the CatS constructs were generated by transposition using the Bac-to-Bac system (Invitrogen). High-titer viral stocks were generated by infection of *Spodoptera frugiperda* Sf9 cells and the expression of recombinant protein was carried out by infection of *Trichoplusia ni* Hi5 cells (Invitrogen) in 10L Wave Bioreactors (Wave Biotech).

[00189] Most of the protein was secreted into the media. The cell supernatant was concentrated, and diafiltered by cross flow ultrafiltration. The protein in the supernatant was purified by passage over ProBond (InVitrogen) resin. After activation was achieved by altering the pH to low pH, E64 was added and cation exchange chromatography was used to isolate the CatS-E64 complex. The CatS (SEQ. ID No. 3) protein purity as determined on denaturing SDS-PAGE gel was 90-95%. CatS (SEQ. ID No. 3) was concentrated to a final concentration of 8.5 mg/ml and stored at 4°C in a buffer containing 25 mM Sodium Acetate, pH 5.5, 150 mM NaCl, 1.5 mM benzamidine and a five-fold molar excess of E64.

[00190] This example describes the crystallization of the complex of CatS (SEQ. ID No. 3)- with E64 ~~complex~~. It is noted that the precise crystallization conditions used may be further varied, for example by performing a fine screen based on these crystallization conditions.

[00191] Residues 114-331 of CatS with a C-terminal Glycine-6x-histidine tag (SEQ. ID No. 3) protein samples were incubated with 1.5mM benzamidine and a fivefold molar excess of E64 before setting crystallization trials. Crystals were obtained after an extensive and broad screen of conditions, followed by optimization. Diffraction quality crystals were grown as in 100nL sitting droplets using the vapor diffusion method. 50nL comprising the CatS-E64 complex (8.5 mg/ml) was mixed with 50nL from a reservoir solution (100μL)

comprising 0.2M citrate/citric acid pH=5.0, 16% (w/v) PEG 4000 and 5% (w/v) PEG 200.
The resulting solution was incubated over a period of one week at 4°C.

Please amend the headings to Tables 2-8 at pages 14-16 as follows:

Table 2: The catalytic domain of CatS (residues 115-331 of SEQ. ID No. 1) binding site residues within 4 Angstroms of E64.

Table 3: The catalytic domain of CatS (residues 115-331 of SEQ. ID No. 1) binding site residues within 7 Angstroms of E64.

Table 4: The catalytic domain of CatS (residues 115-331 of SEQ. ID No. 1) binding site residues within 10 Angstroms of E64.

Table 5: The catalytic domain of CatS (residues 115-331 of SEQ. ID No. 1) binding site residues within 4 Angstroms of Trp186.

Table 6: The catalytic domain of CatS (residues 115-331 of SEQ. ID No. 1) binding site residues within 7 Angstroms of Trp186.

Table 7: The catalytic domain of CatS (residues 115-331 of SEQ. ID No. 1) binding site residues within 4 Angstroms of Ser213.

Table 8: The catalytic domain of CatS (residues 115-331 of SEQ. ID No. 1) binding site residues within 7 Angstroms of Ser213.